COMPARATIVE CLINICAL EVALUATION OF CORONALLY ADVANCED FLAP (CAF) WITH OR WITHOUT PLATELET-RICH FIBRIN (PRF) MEMBRANE IN THE TREATMENT OF ISOLATED GINGIVAL RECESSION – 6 MONTHS RANDOMIZED CONTROLLED STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II

PERIODONTICS

APRIL 2012

CERTIFICATE

This is to certify that **Dr. M. Thamaraiselvan**, Postgraduate student in the Department of Periodontics, J.K.K.Nattraja Dental College and Hospital, Komarapalayam has done this dissertation titled **"COMPARATIVE CLINICAL EVALUATION OF CORONALLY ADVANCED FLAP (CAF) WITH OR WITHOUT PLATELET-RICH FIBRIN (PRF) MEMBRANE IN THE TREATMENT OF ISOLATED GINGIVAL RECESSION – 6 MONTHS RANDOMIZED CONTROLLED STUDY"** under my direct guidance during his post graduate study period 2009 -2012.

This dissertation is submitted to **THE TAMILNADU Dr. MGR MEDICAL UNIVERSITY,** in partial fulfillment of the degree of **MASTER OF DENTAL SURGREY, BRANCH II – Periodontics.**

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ACKNOWLEDGEMENT

*I am greatly indebted to my teacher **Dr. Sugumari Elavarasu**, Professor and Head, Department of Periodontics, J.K.K.Nattraja Dental College and Hospital, Komarapalayam, for her strenuous and dedicated efforts towards the post graduate students and for her invaluable guidance, support and encouragement throughout my post graduate study.*

*I would like to extend my heartfelt gratitude to the Professor **Dr.A.Sivakumar,** Principal, J.K.K.Nattraja Dental College and Hospital, for his kindness in allowing me to utilize the facilities in the college.*

*It is with a deep sense of respect that I express my sincere and heartfelt gratitude to my teacher, **Dr.Abinaya**, Professor, for her patient approach, constant encouragement and guidance throughout my post graduate study.*

*I pay my regards and immense respect to my esteemed sir **Dr.S.Thangakumaran**, Reader. His vast and up-to -date knowledge in the subject has always proven to be a boon for his students. I profoundly thank him for his invaluable guidance, constructive criticism and the liberty he provided me to carry out this dissertation.*

*I express my earnest regards to **Dr. G.S. Jayaprakash,** Senior Lecturer, for his inestimable suggestions, timely advice and encouragement he gave me during this study. I will forever be indebted to him for his overwhelming benevolence and kindness he has shown me in times of need.*

*My sincere thanks to my senior postgraduates, **Dr. Arthi**, Senior Lecturer and **Dr. Sasi Kumar,** Senior lecturer, who constantly supported and cheered me at every*

step of this study. I also wish to gratefully acknowledge my fellow & junior postgraduate colleagues ***Dr. Ria Maria, Dr. Pirinitha, Dr. Devisree, Dr. Santhosh****,* ***Dr. Vennila*** *for their criticism and continued support throughout my post graduate study.*

My heartful thanks to ***Mrs.Vijalakshmi*** *and* ***Mr.Sakthivel*** *librarian and all non-teaching staff' for their kind help during my postgraduate period.*

I would like to thank all my ***patients*** *for their kind cooperation.*

I bow my head in reverence to my parents, ***Mr. A. Murugan*** *and* ***Mrs. Devaki,*** *who have overcome innumerable obstacles with forbearance, enabling me to get this far.*

Above all, I wish to thank and praise the ***LORD*** *for bestowing his grace and mercy that have given me strength, hope and sustenance throughout.*

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Gingival recession is defined as the displacement of gingival margin apical to cemento-enamel junction (CEJ).[1] It results in higher incidence of attachment loss, root caries, hypersensitivity and esthetic concerns.[2] Different factors like high muscle attachment or frenal pull, alveolar bone dehiscence, tooth malposition and traumatic tooth brushing have been related to the development of gingival recession.[3]

Since mid 1950 various periodontal plastic surgical procedures were developed and have shown predictable results in correcting gingival recession defects. Traditional approaches like free gingival grafts (FGG), sub-pedicle and sub-epithelial connective tissue grafts (SCTG) requires the harvesting second surgical site, which often resulted in post operative complications like pain, persistent bleeding and secondary healing at the donor site.[4]

Coronally advanced flap (CAF) is a relatively simple, pedicle flap procedure that offers several advantages like predictable root coverage, better colour and contour match.[5] But it heals with long junctional epithelium[6] which results in more chances of recurrence.

The Guided Tissue regeneration (GTR) procedure were also used in the treatment of gingival recession, with the goal of obtaining both root coverage and new connective tissue attachment.[7] However there are several drawbacks such as technical difficulties in barrier membrane placement, exposure of membrane in the course of healing and colonization of periodontal pathogens on exposed barrier membrane.[4]

The recent introduction of autologous biomimetic agent, Platelet-rich fibrin (PRF) membrane has given a new promise for better outcomes in periodontal regeneration. It represents the second generation platelet concentrate therapeutic system. Unlike other platelet concentrates, PRF requires neither anticoagulant, nor bovine thrombin for platelet activation.[8]

PRF consists of a fibrin three dimensional (3D) matrix, polymerized in a specific structure with the incorporation of platelets, leukocytes, growth factors and circulating stem cells.[9] Though platelet and leukocyte cytokines play an important part in the biology of this material, fibrin matrix supporting them certainly constitutes the determining element responsible for the real therapeutic potential.[8] This fibrin matrix implies an increased lifespan for the cytokines, because they will be released and used only at the time of initial matrix remodeling. Cytokines are thus available in situ for a convenient period of time (long term effect).[8] Moreover its molecular structure with low thrombin concentration is an optimal matrix for migration of endothelial cells and fibroblasts which permits rapid angiogenesis and easier remodeling of connective tissue.[8] Thus PRF is considered as a healing biomaterial which is now used in periodontal plastic and implant surgical procedures to enhance bone regeneration and soft tissue wound healing.

The present study was conducted to clinically evaluate the effectiveness of autologous PRF membrane with CAF in the treatment of isolated gingival recession compared to CAF alone.

The aim of the present study was to clinically evaluate the effectiveness of autologous PRF membrane with CAF in the treatment of isolated gingival recession compared to CAF alone, based on the following parameters

- Amount of Mean root coverage (MRC) obtained in terms of recession depth.
- Changes in Clinical attachment level (CAL).
- Changes in Width of keratinized tissue (WKT).
- Changes in Gingival thickness (GTH).

Gingival recession is a term that designates the oral exposure of root surface because of the displacement of the gingival margin apical to the cemento enamel junction.[10] Tissue trauma caused by vigorous tooth brushing is considered to be one of the predominant causative factors for development of recession, particularly in young adults. Other local factors are alveolar bone dehiscence, high muscle attachment or frenal pull, plaque and calculus, iatrogenic factors related to restorative and periodontal treatment procedures.[11]

Inflammation plays a role in the pathogenesis of recession, where it is essential for the formation of cleft defects.[12] The clefts were claimed to be due to the growth and anastomosis of retepegs of the oral epithelium and the epithelium lining the periodontal pocket.[12] It is possible that overzealous tooth brushing could lead to a subclinical inflammation by increasing the epithelial permeability.[12]

Width of attached gingiva and periodontal health

For many years, the presence of adequate zone of gingiva was considered critical for the maintenance of marginal tissue health and for the prevention of continuous loss of connective tissue attachment.[13] Initial cross sectional studies showed that a correlation exists between the presence of narrow zone of gingiva and the development of gingival recession.[14]

A few longitudinal studies have been reported, in which the role of a zone of keratinized or attached gingiva for the long term maintenance of the attachment level has been properly assessed.

Lindhe and Nyman et al. (1980)[15] examined the alteration of the position of the gingival margin in relation to CEJ, following periodontal surgery in patients with advanced periodontal breakdown. During the maintenance care of 10 to 11 years, no recession was observed, both in areas with and without keratinized tissue.

Dorfman et al. (1982)[16] in a 4 year longitudinal study reported that no further recession of the gingiva or loss of probing attachment had occurred in areas where there is lack of firmly attached marginal soft tissue. It was concluded that sites without attached gingiva might not experience further attachment loss and recession if inflammation is controlled.

Kennedy et al. (1985)[17] evaluated the patients, who had not participated in maintenance program for a period of 5 years. Except for the clinical signs of inflammation which was more pronounced on grafted sites, no difference were observed between sites with less than 1 mm or complete lack of attached gingiva and grafted sites.

The lack of relationship between the width of attached gingiva and the development of soft tissue recession is validated from these longitudinal studies. These prospective longitudinal studies show that gingival height is not a critical factor for the prevention of marginal tissue recession.

Root coverage

The main indications for root coverage procedures are esthetic/ cosmetic demand, root sensitivity, changing the topography of the marginal soft tissue in order to facilitate plaque control.[18] A variety of root coverage surgical techniques are available, which can be grouped into pedicle flaps and free soft tissue grafts.[19]

Grupe and Warren et al. (1956)[20] proposed the first method for covering a localized gingival recession with laterally sliding flap (LPF) operation. The tooth adjacent to the defect serves as the donor site for the flap that is moved laterally to cover the defects. The major limitations are the development of recession at the donor site, technique is limited to site with an adequate amount of adjacent keratinized tissue.[21]

Double papilla flap a variant of laterally positioned flap utilizes the adjacent papilla and moves it to the mid-facial area. This eliminates the risk of facial recession on the adjacent tooth and works best when the donor papilla is wide.[21] It often gives poor results, because the blood supply is impaired by suturing two flaps over the root surface.[21] Unfortunately no studies have examined the predictability of this technique. This may be due to the limited indications for their use.[21]

Free gingival grafts (FGG) were also utilized for root coverage procedures, which heals by bridging and creeping attachment.[22] Greater predictability was established when a thick graft of atleast 2mm was used. The major disadvantages of this procedure are a large, slow healing donor site, often an unfavorable color match and root coverage rarely complete and not entirely predictable.[22]

Coronally advanced flap (CAF)

The coronal advanced flap was first introduced by Norberg (1926) as an aesthetic surgical procedure for root coverage. In 1958, Patur and Glickman stated that the coronal advancement of pedicle flap was not an effective means of covering exposed root surface since it requires the presence of residual keratinized tissue with the same height of the depth of recession.

Harvey et al. (1970)[23] was probably the first to propose a composite procedure including, the establishment of a wider zone of attached gingiva with FGG, following 6 months later by a coronally positioned flap to cover the recession.

Bernimoulin et al. (1975)[24] in a 1 year study evaluated the results of coronally repositioned flap as proposed by Harvey et al. (1970). The results showed that the CPF consistently resulted in a significant reduction of recession. In addition to this there were no significant differences between reattachment values at 1, 6, and 12 months postoperatively.

Matter J (1979)[22] reported a case series in which 11 cases with 36 recession defects were treated to increase the width of attached gingiva by a free gingival graft, following 2 months later by a coronally repositioned flap. The results showed that in all cases the procedure has much improved the periodontium functionally as well esthetically.

Allen EP and Miller PD (1989)[25] reported the short-term results in the treatment of shallow marginal tissue recession using coronal positioning of the existing gingiva. The results showed that a 97.8 % root coverage at 6 months postoperatively. Complete root coverage was attained in 84 % of the treated sites.

Pini Prato G et al. (1999)[26] designed a prospective clinical, controlled randomized study to determine if mechanical instrumentation (Root planing) of the exposed root is useful in treating gingival recession using CAF. After a follow-up of 3 months the result showed that root planing is not necessary when shallow recession are treated using CAF in patients with high level of oral hygiene.

Baldi C et al. (1999)[27] conducted a study to determine whether the flap thickness can influence root coverage using a CAF. After flap elevation and before suturing, the flap thickness was measured using a gauge. With 3 months follow up, the results showed that, a flap thickness of > 0.8 mm was associated with 100% of root coverage and there is a direct relation between flap thickness and recession reduction.

Pini Prato G et al. (2000)[28] measured the tension of the CAF to compare the recession reduction achieved in flap with tension and in flap without tension. After 3 months follow up, the results showed that the higher flap tensions (4 to 11 grams) of the CAF before suturing were associated with a lower recession reduction and minimal flap tensions (0 to 4 grams) were often associated with complete root coverage.

Saletta D et al. (2001)[29] evaluated the dimension of the interdental papilla, as a prognostic factor for the clinical outcome of the CAF in the treatment of gingival recession. They indicated that the root coverage following CAF procedure is not significantly correlated to the interdental papilla area or to the papilla height. However complete root coverage is significantly more frequent in sites with lower height of the interdental papilla.

Pini Prato G et al. (2005)[30] investigated whether the post-surgical location of the gingival margin relative to cemento-enamel junction (CEJ) can influence the recession reduction and complete root coverage following CAF procedure. Results showed that the recession depth at baseline (REC_{T0}) & the location of gingival margin after suturing (GM_1) are positively correlated to recession reduction. There was greater probability of complete root coverage when the gingival margin was placed at more coronal level after suturing (GM_1). It was concluded that the location of the gingival margin relative to the cemento-enamel junction following CAF procedure seems to influence complete root coverage.

Zucchelli G et al. (2009)[31] compared the root coverage and esthetic outcomes of the CAF with and without vertical releasing incisions in the treatment of multiple gingival recessions in a controlled randomized clinical trial. After one year follow-up the results showed that, the envelope-type of CAF was associated with an increased probability of achieving complete root coverage and with a better post-operative course. Keloid formation along the vertical releasing incisions was responsible for the worst esthetic outcome.

Zucchelli G et al. (2009)[32] in a randomized controlled split-mouth clinical trial evaluated the effectiveness of hand and ultrasonic root instrumentation, in combination with a CAF for the treatment of isolated type recession defects. The results obtained failed to demonstrate any superiority, in terms of root coverage, for hand instrumentation over ultrasonic treatment of the root surface in combination with CAF surgery.

Santana RB et al. (2010)[33] designed a study to compare the clinical outcomes of the semilunar coronally re-positioned flap (SLCRF) & CAF procedure in the treatment of maxillary recession defects. The CAF resulted in clinical improvements significantly better than SLCRF for percentage of root coverage, frequency of complete root coverage (CRC) & CAL gain. It was concluded that recession coverage is significantly better with CAF compared with the original SLCRF technique.

Pini Prato G et al (2011)[34] in a long term 14 year-randomized split-mouth study evaluated the 1) outcomes of two different methods of root surface modification used in combination with a CAF and 2) the long term results of CAF performed for the treatment of single gingival recessions. At 14 years, recession increased slightly over time in both groups. The result showed that during a long term follow-up, gingival recession recurred in 39 % of the treated sites following the CAF procedure.

Coronally advanced flap with subepithelial connective tissue graft

Silva RCD et al. (2004)[35] in a randomized clinical trial compared the CPF alone or in conjunction with a subepithelial connective tissue graft in the treatment of gingival recession. The results indicated that both surgical approaches are effective in addressing root coverage. The authors concluded that, when an increase in gingival dimensions is a desired outcome, then the combined technique may be used.

Pini-prato GP et al (2010)[36] in a long term study compared the clinical outcome of CAF alone versus CAF plus connective tissue graft (CTG) in the treatment of multiple gingival recessions using split-mouth design over a 5 years follow-up period. At 5 years, CAF + CTG treated sites showed high percentage CRC than CAF alone treated sites. Apical relapse of the gingival margin was observed in CAF sites; while a coronal improvement of margin was noted in CAF + CTG treated sites between 6 months and 5 years follow-up. It was concluded that CAF + CTG provided better CRC than CAF alone in the treatment of multiple gingival recession at the 5 year follow-up.

Coronally advanced flap with Acellular dermal matrix

Cortes ADQ et al. (2004)[37] clinically evaluated the treatment of class I gingival recessions by CPF with or without acellular dermal matrix allograft (ADM). The authors concluded that both techniques may provide significant root coverage in class I gingival recessions; however, a greater keratinized tissue thickness can be expected with ADM.

Woodyard JG et al. (2004)[38] in a randomized, blinded, controlled clinical investigation compared the coronally positioned flap (CPF) plus an acellular dermal matrix (ADM) allograft to CPF alone to determine their effect on gingival thickness and percentage root coverage. The results showed that the treatment with CPF plus an ADM allograft significantly increased gingival thickness when compared to CPF alone. Recession defect coverage was significantly improved with ADM.

Coronally advanced flap with barrier membrane

Amarante ES et al. (2000)[39] compared the clinical outcome following treatment of gingival recession by CAF procedure alone, or combined with a bioresorbable membrane using a split mouth blinded randomized study. After a 6 months follow up, the results showed that CAF operation offers a predictable, simpler and convenient approach as a root coverage procedure in recession defects. Combining this technique with the placement of a bioresorbable membrane does not seem to improve the results.

Lins LHS et al. (2003)[40] evaluated the clinical outcome of root coverage using coronally positioned flap (CPF) with and without Guided tissue regeneration (GTR) using titanium reinforced expanded polytetrafluoro ethylene (ePTFE) barrier. Results after 6 months showed both GTR & CPF procedure resulted in root coverage. The amount of root coverage obtained with CPF was greater than that observed with GTR, although GTR resulted in significantly greater alveolar crest level (ACL) gain.

Leknes KN et al. (2005)[41] evaluated the clinical outcome of CAF procedure with or without a biodegradable membrane in the treatment of human gingival

recession in a randomized controlled split-mouth clinical trial. It was concluded that placement of a biodegradable membrane underneath the flap does not seem to improve neither the short- nor the long-term results.

Coronally advanced flap with Emdogain

Modica F et al. (2000)[42] evaluated the effect of emdogain when combined with CAF. Six months post surgery the results suggested that emdogain does not seem to significantly improve the clinical outcome of gingival recession treated by means of CAF, even though the test group showed slightly better results in terms of root coverage and clinical attachment level.

McGuire MK and Nunn M (2003)[43] designed a study to compare the clinical efficacy of enamel matrix derivative (EMD) placed under a CAF with subepithelial connective tissue graft (SCTG) placed under a CAF in patients with recession defects. Clinical parameters were measured at baseline & at 6, 9, & 12 months. The results obtained suggested that the addition of EMD to the CAF resulted in root coverage similar to the subepithelial connective tissue graft but without the morbidity & potential clinical difficulties associated with the donor site surgery.

McGuire MK and Nunn M (2003)[44] compared the histological examination of the subepithelial connective tissue graft (SCTG) and EMD plus CAF. A patient presented with two hopeless teeth was randomized to receive SCTG or a CAF plus EMD. The treated teeth and a small collar of tissue were removed at 6^{th} month and underwent histological analysis. Histologic examination revealed, SCTG adhere to the root surface primarily by a connective tissue attachment with some evidence of

root resorption. The CAF with EMD was found to have all the tissues necessary for regeneration: new cementum, organizing periodontal ligament (PDL) fibers and islands of condensing bone. These histologic sections strongly suggest that enamel matrix derivative works in a biomimetic fashion by mimicking the natural process of tooth development.

Pilloni A et al. (2006)[45] designed a study to examine the effects of EMD combined with the CPF over an 18-month postoperative period. The results of this study indicated that topical application of EMD is beneficial in augmenting the effects of the CPF in terms of amount of root coverage; gain in clinical attachment, and in increasing the apicocoronal dimension of the keratinized tissue.

Growth factors

The lack of a predictable outcome when using passive therapies, such as osteoconductive matrices and guided tissue regeneration, led to the development of treatments designed to stimulate the cells responsible for regeneration.[46] The tissue engineering combines three key elements to enhance regeneration: conductive scaffolds, signaling molecules and cells.[46] The important biological event involved in tissue regeneration is specific cell directed migration.[46] A variety of naturally occurring potent bioactive proteins are known to be present in bone, platelets, and a number of other cells and tissues and these regulates events in tissue engineering.[47]

Polypeptide growth factors (PGF) are naturally occurring biological modifiers that have the potential to alter the host tissue to stimulate or regulate the wound healing process. They regulate key cellular events in tissue regeneration, including

cell proliferation, chemotaxsis, differentiation, and matrix synthesis via binding to specific cell surface receptors.[47] Growth factors either singly or in combination have been used and experimental evidence for regeneration has been documented in both animal and human trials.[48]

Platelet Rich Plasma (PRP)

PRP is procured from whole blood and is rich in platelets and naturally occurring autologous growth factors that are present in plasma.[49] Its use is based on the potential of the plasma to release multiple wound-healing growth factors and cytokines, which are responsible for increasing cell mitosis, increasing collagen production, recruiting other cells to the site of injury, initiating vascular in-growth and inducing cell differentiation.[50]

In-vitro studies

Kawase T et al. (2003)[51] designed a study to investigate PRPs action on extracellular matrix production in periodontal ligament (PDL) and osteoblastic MG63 cell cultures. The results showed that the PRP changed cell shape and up-regulated type I collagen. Fibrinogen was detected in the PRP preparations and insoluble fibrin networks were found. The authors suggested the possibility that fibrinogen, converted to fibrin, in combination with growth factors present in PRP and might effectively promote wound healing at sites of injury in periodontal tissue.

Uggeri J et al. (2007)[52] investigated the dose-dependent effects of platelet gel releasate (PGR) on activities of human osteoblasts. The results showed that the PGR stimulated osteoblast proliferation in a dose-dependent manner and, when used at

33% and 11%, induced maximum levels of ALP and collagen synthesis. Moreover, in the presence of dexamethasone (dex) and β-glycerophosphate (β-GP), PGR stimulated the end maturative status of cells as expressed by the deposition of calcium nodules.

In-vivo studies

Cheung WS and Griffin TJ (2004)[2] in a randomized clinical trial assessed the clinical efficacy of Platelet concentrate grafts (PCG) in the treatment of gingival recession and compared their soft tissue healing with those of subepithelial connective tissue graft (SCTG). The results showed no statistically significant differences between the treatments groups. It was concluded that the platelet concentrate graft may be an alternative graft material for treating gingival recession. Treatment with this graft may result in better esthetic appearance.

Huang LH et al. (2005)[53] conducted a pilot human trial to evaluate the effects of PRP in combination with CAF in the treatment of gingival recessions. Based on the results it was concluded that, the application of PRP in CAF root coverage procedure provides no clinically measurable enhancements on the final therapeutic outcomes.

Keceli HG et al. (2008)[54] designed a study to compare connective tissue graft (CTG) and PRP with CTG alone in the treatment of gingival recession. Based on the results, the authors concluded that no difference could be found between CTG and CTG with PRP group.

Shepherd N et al. (2009)[55] in a pilot study compared the percentage of recession defect coverage obtained with a coronally positioned tunnel (CPT) plus an ADM to that of a CPT plus ADM and platelet rich plasma (CPT/PRP) 4 months post-surgically. The results showed that the CPT plus ADM and PRP produced defect coverage of 90% whereas the CPT with ADM produced only 70% defect coverage. It was concluded that the difference was not statistically significant, but it may be clinically significant.

Platelet Rich Fibrin (PRF)

Choukroun's Platelet-rich fibrin (PRF) is a second generation platelet concentrate, which was first developed by ***Choukroun et al. (2001)***[56] in France. It is defined as an autologous Leukocyte and Platelet-rich fibrin (L-PRF) biomaterial.[9, 57] Unlike other platelet concentrates, PRF requires neither anticoagulants nor bovine thrombin (nor any other gelling agent).[58] PRF results from a natural and progressive polymerization occurring during centrifugation.[58] Its production protocol attempts to accumulate platelets, leukocytes and their released cytokines in a three dimensional fibrin network.[9,57]

Role of platelet cytokines in PRF

Platelet cytokines like Transforming Growth Factor-β (TGF-β), Platelet Derived Growth Factor (PDGF), Insulin like Growth Factor (IGF) are trapped in the fibrin meshes of PRF, and probably even in the fibrin polymers during polymerization.[9] These cytokines play a fundamental role in initial tissue healing mechanisms, owing to their capacity to stimulate cell migration and proliferation

(primarily by PDGF), induced fibrin matrix remodeling as well as secretion of collagen matrix (primarily by TGF-β) and as cell protective agents (primarily by IGF).[9] PRF also enmeshes glycosaminoglycans (heparin and hyaluronic acid) from blood and platelets. They have a strong affinity with small circulating peptides such as platelet cytokines and a great capacity to support cell migration and healing processes.[9]

Role of leukocytes in PRF

A high number of leukocytes are concentrated in one part of the dense fibrin matrix of PRF during polymerization.[57] These leukocytes seem to have a strong influence on growth factors release, immune regulation, anti-infectious activity and matrix remodeling during healing.[57,59] Its defense capacities against infections would be quite significant, by the chemotactic properties of cytokines as well as by their ability to facilitate, access to the injured site.[57] Thus PRF could be considered as an immune organizing node.

Role of fibrin matrix in PRF

Though platelets and leukocyte cytokines play an important part in the biology of this biomaterial, the fibrin matrix supporting them certainly constitutes the determining element responsible for the real therapeutic potential of PRF.[8] Its molecular structure with low thrombin concentration is an optimal matrix for migration of endothelial cells and fibroblasts.[8] It permits a rapid angiogenesis and an easier remodeling of fibrin.[8] During healing the fibrin matrix traps the circulating stem cells brought to the injured site.[8] These undifferentiated cells are able to

differentiate into several different cell types necessarily in the presence of fibrin and fibronectin.[8] Based on this several author's have demonstrated that, fibrin matrix is an optimal support to transported mesenchymal stem cells for obtaining regeneration.[8,60] Moreover a progressive polymerization mode signifies an increased incorporation of the circulating cytokines in the fibrin meshes (intrinsic cytokines).[9] It was hypothesized that the natural fibrin framework of PRF can protect the growth factors from proteolysis, thus its activity is retained for a relatively longer period of time and stimulate regeneration effectively.[61]

In-vitro studies

Dohan DM et al. (2006)[9] carried out a comparative study to quantify PDGF-BB, TGF-β1 and IGF-1 within PPP (platelet-poor plasma) supernatant and PRF clot exudate serum. The results revealed that slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in fibrin meshes. It was concluded that PRF, unlike other platelet concentrates, would be able to progressively release cytokines during fibrin matrix remodeling.

Dohan DM et al. (2006)[57] investigated the immune features of PRF by quantifying 5 significant cell mediators within platelet poor plasma supernatant and PRF clot exudate serum: 3 proinflammatory cytokines (IL-β, IL-6, and TNF-α), an anti-inflammatory cytokine (IL-4), and a key growth promoter of angiogenesis (VEGF). The results revealed that PRF could be an immune regulation node with inflammation retrocontrol abilities.

He L et al. (2009)[61] evaluated the effect of biologic characteristics of PRP and PRF on proliferation and differentiation of rat osteoblasts. The results showed that the PRF released autologous growth factors gradually and expressed stronger and more durable effect on proliferation and differentiation of rat osteoblast than PRP in vitro.

Su CY et al. (2009)[62] studied the in vitro release of growth factors from PRF and supernatant serum to optimize clinical use. The results showed that the growth factors were also found in serum supernatant. Protein profiles of the releasates and the supernatant serum were similar. It was concluded that the PRF membrane should be used immediately after formation to maximize the release of growth factors to the surgical site. The remaining fluid can be recovered as an additional source of growth factors for grafting.

Dohan Ehrenfest DM et al. (2009)[63] analysed the affects of Choukroun's PRF, on human primary cultures of gingival fibroblasts, dermal prekeratinocytes, preadipocytes and maxillofacial osteoblasts. The results showed that the PRF induced a significant and continuous stimulation of proliferation of all cell types. Moreover PRF induced a strong differentiation in the osteoblasts.

Dohan Ehrenfest DM et al. (2010)[64] analysed the in vitro effects of PRF on human bone mesenchymal stem cells (BMSC), harvested in the oral cavity after preimplant endosteal stimulation. PRF generated significant stimulation of the BMSC proliferation and differentiation which was dose dependent. It was concluded that this double contradictory proliferation/differentiation result may be due to the

numerous components of PRF, particularly the presence of leukocytes. It could be the source of differential geographic regulation processes within the culture.

Gassling V et al. (2010)[60] compared PRF membrane with the commonly used collagen membrane Bio-Gide® as scaffold for periosteal tissue engineering. From the results obtained it was concluded that PRF appears to be superior to collagen (Bio-Gide®) as a scaffold for human periosteal cell proliferation. Thus PRF membranes are suitable for in vitro cultivation of periosteal cells for bone tissue engineering.

Dohan Ehrenfest DM et al. (2010)[65] designed a study to perform a detailed examination of the composition and architecture of the choukroun's PRF clot (particularly the distribution of the platelets and leukocytes within the fibrin clot) using hematologic counts, photonic microscopy, and SEM. They also analysed the structural & morphological differences between PRFs commonly produced with two different kinds of collection tubes (dry glass tubes and glass-coated plastic tubes) and using two different methods for the compression of the PRF clot into the membrane (forcibly or softly). Platelet counts clearly showed that there was hardly any platelet left within the red blood cell (RBC) layer, platelet poor plasma (PPP), or the exudate provided by compressing the PRF clot. Leukocyte counts confirmed that more than half of the leukocytes were trapped in PRF membranes, and small lymphocytes seemed mainly collected. The photonic microscopy study showed that the platelets and leukocyte distribution within the clot was not uniform. Platelets and leukocytes were concentrated in an intermediate layer located between RBCs and the fibrin clot and represent a macroscopic buffy coat on the PRF-clot surface. Therefore, the authors suggested that, when harvesting clots for surgical use, practitioners should

collect this intermediate whitish layer. Thus, it is necessary to preserve a small RBC layer at the PRF clot end to collect as many platelets and leukocytes as possible. SEM evaluation showed that RBCs were widely predominant in the red part of the PRF clot, and the leukocytes were distributed at the junction between the red and yellow parts of the clot. Platelet morphology is totally modified by aggregation and clotting processes. Therefore it was not possible to identify non-activated platelets (discoid bodies) but rather only a large aggregate of platelet-fibrin polymers.

In-vivo studies

Choukroun J et al. (2006)[66] evaluated the potential of PRF in combination with freeze-dried bone allograft (FDBA) (Phoenix; TBF, France) to enhance bone regeneration in sinus floor elevation. It was concluded that sinus floor augmentation with FDBA and PRF leads to a reduction of healing prior to implant placement. From a histologic point of view, this healing time could be reduced to 4 months, but large-scale studies are still necessary to validate these first results.

Diss A et al. (2008)[67] in a prospective study evaluated the radiographic changes in the apical bone levels on microthreaded implants placed in subsinus residual bone height, according to a bone-added osteotome sinus floor elevation (BAOSFE) technique with PRF as grafting material. Results showed that (BAOSFE) procedure with PRF as grafting material can lead to an endosinus bone gain. Despite a limited residual bone height, a healing period of 2-3 months was found to be sufficient to resist a torque of 25 N.cm applied during abutment tightening. At 1 year,

formation of new recognizable bone structure delimiting the sinus floor was identified radiographically.

Simonpieri A et al. (2009)[68] described the implant and prosthesis phases of a complex maxillary rehabilitation, after periimplant bone grafting using allograft, Choukron's PRF and metronidazole. It was concluded that PRF membrane are particularly helpful for periosteum healing and maturation. The thick periimplant gingiva is related to several healing phases of a PRF membrane layer and could explain the low marginal bone loss observed.

Anilkumar K et al. (2009)[69] reported a case of root coverage in mandibular anterior teeth using PRF with laterally displaced flap technique. The result showed no post-operative complications and the healing was satisfactory. Complete root coverage was achieved six months after the procedure, with excellent tissue contour and esthetics.

Aroca S et al. (2009)[70] designed a study to determine whether the addition of an autologous PRF clot to a modified coronally advanced flap (MCAF) (test group) would improve the clinical outcome compared to an MCAF alone (control group) for the treatment of multiple gingival recessions. The results concluded that MCAF is a predictable treatment for multiple adjacent recession defects. The addition of a PRF membrane positioned under the MCAF provided inferior root coverage but an additional gain in gingival thickness at 6 months compared to conventional therapy.

Jankovic S et al. (2010)[71] evaluated the clinical effectiveness of PRF membrane used in combination with a CAF and to compare it with the use of an EMD in combination with a CAF in gingival recession 12 months post treatment. The results demonstrated that there was no clinical advantage in the use of PRF compared to EMD in the root coverage of gingival recession with CAF procedure. The EMD group showed a higher success rate in increasing width of keratinized tissue than did the PRF group.

Sharma A and Pradeep AR (2011)[72] in a double-masked randomized study evaluated the effectiveness of autologous PRF in the treatment of mandibular degree II furcation defects compared with open flap debridement. Results showed a significant improvement at the sites treated with PRF and OFD compared to those with OFD alone. It was concluded that, the improvement with autologous PRF implies its role as a regenerative material in the treatment of furcation defects.

A randomized controlled clinical study was conducted to evaluate the effectiveness of autologous PRF membrane with CAF in the treatment of isolated gingival recession compared to CAF alone. The protocol was reviewed and approved by institutional ethical board. The study related procedures were explained to the patients before they sign an informed consent form. A total of 20 subjects (18 males, 2 females) each with one buccal recession defects were recruited from the outpatient Department of Periodontics, J. K. K. Nattraja Dental College and Hospitals, Komarapalayam, Tamilnadu based on the following criteria.

Inclusion criteria[53]

1. Maxillary or mandibular incisors, canines, or premolars with Miller's class I or II (confirmed by radiographic analysis of involved tooth) recession defect.
2. Age above 18 years.
3. Ability to maintain good oral hygiene (full-mouth plaque index < 20%).
4. Systemically healthy subjects.

Exclusion criteria[70]

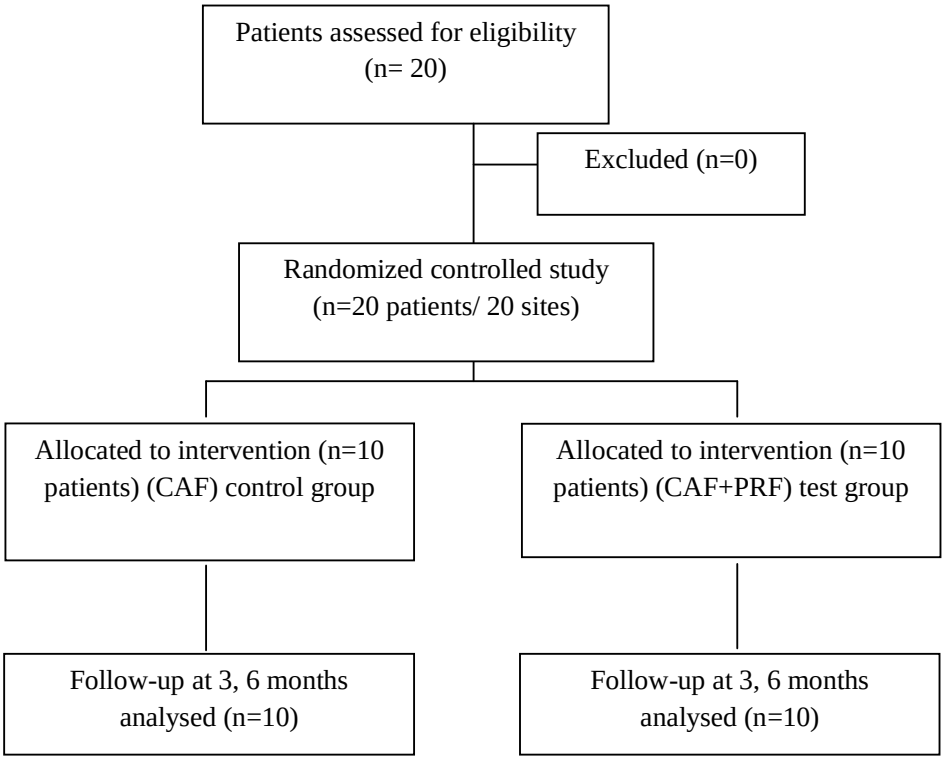
1. Previous surgical attempt to correct the gingival recession.
2. Presence of inflammatory periodontal disease.
3. Patients under anticoagulation treatment.
4. Pregnant and lactating women.
5. Smokers.
6. Caries or restorations in the area to be treated.

Study Design

A randomized controlled clinical study was designed, in which the 20 subjects were randomly assigned to two treatment groups by drawing envelops including notes stating either test or control.

- Control group included 10 Miller's class I or II defects treated with CAF.
- Test group included 10 Miller's class I or II defects treated with CAF + PRF.

Diagram of study design



Clinical parameters[53]

The following clinical parameters were recorded at baseline (BL), 3rd month and 6th month. All linear measurements were rounded to the nearest 0.5 mm using a William's periodontal probe.

- Probing depth (PD) was measured at mid-buccal point on the custom stent. The measurement was made from free gingival margin to the most apical part of sulcus.

- Clinical attachment level (CAL) was measured at mid-buccal point on the custom stent. The measurement was made from the cemento enamel junction (CEJ) to the most apical part of the sulcus.

- Recession depth (RD) was measured at the mid-buccal point on the custom stent. The measurement was made from the CEJ to the free gingival margin.

- Recession width (RW) was measured at the CEJ of the crown in a mesio-distal direction.

- Width of keratinized tissue (WKT) was measured at the mid-buccal point from the mucogingival junction to the free gingival margin. The mucogingival junction was determined using the rollover technique, where in the mucosa was rolled until the nonmovable portion of the attached keratinized tissue was identified.

- Gingival thickness (GTH) was measured 3 mm below the gingival margin at the attached gingiva or the alveolar mucosa using a # 15 endodontic reamer

with a silicon disk stop. The mucosal surface was pierced at a 90 degree angle with slight pressure until hard tissue was reached. The silicon stop on the reamer was slid until it was in close contact with the gingiva. After removal of the reamer, the distance between the tip of the reamer and the inner border of the silicon stop was measured to the nearest millimeter using a caliper with 0.1 mm calibration.[70]

- Plaque index (PI) was recorded according to ***Silness and Loe 1964.***

The oral hygiene status was evaluated by the presence or absence of visible plaque present at the soft tissue margin. The areas examined were distofacial, facial, mesiofacial and lingual surface, using explorer.

Score 0 - No plaque in the gingival area.

Score 1 - A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be recognized only by running a probe across the tooth surface.

Score 2 - Moderate accumulation of soft deposits within the gingival pocket & on the gingival margin or adjacent tooth surface that can be seen by the naked eye.

Score 3 - Abundance of soft matter within the gingival pocket or on the gingival margin & adjacent tooth surface.

The plaque score per tooth was obtained by totaling the four plaque scores per tooth and then divided by four. The plaque score per person is

obtained by adding the plaque score per tooth and dividing by the number of teeth examined.

The scoring criteria are as follows

0.1 -1.7 - Good.

1.8 - 3.4 - Fair.

3.5 – 5.0 - Poor.

- Gingival index (GI) was recorded according to ***Loe and Silness 1963***.

The soft tissue surrounding each tooth were divided into 4 gingival scoring units i.e. the distofacial papilla, the facial margin, the mesiofacial papilla and the entire lingual margin. A periodontal probe was used to assess the bleeding of the gingival tissues on probing.

Gingival units were assessed according to the following criteria:

Score 0 - Normal gingiva

Score 1 - Mild inflammation, slight change in color, slight edema, no bleeding on palpation.

Score 2 - Moderate inflammation, redness, edema & glazing, bleeding on probing.

Score 3 - Severe inflammation, marked redness & edema, ulceration, tendency for spontaneous bleeding.

The gingival index score for each of the 4 gingival surfaces was given a score from 0 to 3. The scores around each tooth were totaled and divided by four and the gingival index score for each tooth was obtained.

The scoring criteria are as follows

0.1 – 1.0 -Mild

1.1 – 2.0 -Moderate.

2.1 - 3.0 - Severe.

- Percentage of mean root coverage (MRC %)[53,54] was calculated as ([RD preoperative – RD postoperative]/ RD preoperative) x 100 %.

Data collection[53]

Measuring stents for each surgical site was fabricated from self curing acrylic resin. Clinically reproducible measuring points were marked on mid-buccal aspects as standardized reference points to assess clinical parameters.

Presurgical Therapy[53]

For all the enrolled patients routine radiographic and blood investigations were done. The initial therapy consisted of oral hygiene instructions, scaling and root planing and occlusal adjustments as indicated. Three weeks following phase I therapy, a periodontal re-evaluation was performed.

PRF Preparation

The PRF was prepared following the protocol developed by ***Choukroun et al. (2001).***[56] Just prior to surgery, 10 ml of intravenous blood (by a venipuncture of the antecubital vein) was collected in test tubes without anticoagulant and immediately centrifuged at 3000 revolutions (400 g) per minute for 10 minutes. The fibrin clot formed in the middle part of the tube. The upper part contained supernatant serum, and the bottom part contained the red blood corpuscles (RBC). The fibrin clot was easily separated from the red blood corpuscles base (preserving a small RBC layers) using sterile tweezers and scissors, and placed in a sterile metal cup and was left aside to release their serum slowly into the metal cup (soft exudate extraction).[65]

Surgical protocol

All surgical procedures as well as PRF preparation were done by a single investigator. Both test and control groups were treated with CAF using the technique described by ***Pini-Prato et al. (1999)***[26] except for the placement of PRF in test group. Pre operative oral antisepsis was accomplished using 0.2 % chlorhexidine digluconate solution rinse.[40] The surgical area was anaesthetized using lignocaine 2 % with 1: 100000 epinephrine as a vasoconstrictor. The exposed root surface was scaled and planed utilizing hand and ultrasonic instruments. A fresh tetracycline (125 mg tetracycline/ ml of saline) was prepared and applied to the root surface.[2] An intrasulcular incision was made with a number 15 Bard Parker blade on the buccal aspect of the tooth being treated. This incision was horizontally extended mesio-distally to dissect the buccal aspect of the adjacent papillae, avoiding the gingival

margin of the adjacent teeth. Two oblique releasing incisions were carried out from the mesial and distal extremities of the horizontal incisions beyond the mucogingival junction. A trapezoidal full thickness flap was raised with a periosteal elevator towards the mucogingival junction. Then a partial thickness dissection was carried out apically towards the marginal bone crest, leaving the underlying periosteum in place. A mesio-distal and apical dissection parallel to vestibular lining mucosa was performed to release residual muscle tension and facilitate the passive coronal displacement of the flap. The papillae adjacent to the involved tooth were de-epithelialized. At the test sites, previously prepared PRF membrane was placed over the recession defect, just above the CEJ and held in that position with single independent sling suture. The serum exudate from the fibrin clot was applied over the surgical site. The control groups received no further treatment. Flaps were then coronally advanced, with its margin located on the enamel. Suturing of oblique releasing incision was performed with 4-0 silk suture as described by ***Allen and Miller (1989)***,[25] while the coronal mesial and distal extremities of the flap were secured with 2 single sutures placed in the interdental areas. Gentle pressure was applied at the surgical site with moistened gauze to achieve hemostasis and a close adaptation of the flap to the underlying surface. Periodontal dressing was given.

Post-surgical protocol

All patients were prescribed antibiotics and analgesics along with chlorhexidine 0.2% mouthrinse. Post surgical instructions **(Appendix-1)** were given to the patient and recalled after one week for suture removal and follow up.

APPENDIX-1

Post surgical instructions

- Report immediately on development of any untoward reactions like pain, swelling, bleeding, and drug allergies.
- Should avoid intake of any hard & hot foods, not to disturb the operated area with tongue.
- Report if dressing is dislodged.
- Take the prescribed medications regularly as advised.
- Avoid brushing the treated area from the day of surgery, until 2 weeks after suture removal. Use cotton tip applicator (Johnson and Johnson ear buds) to gently clean the area and resume gentle brushing with soft brush and coronally directed roll technique.
- Rinse the mouth with a 0.2 % chlorhexidine solution three times a day for 1 minute for 3 weeks.
- To report as per schedule.

APPENDIX -2

PROFORMA

Name: Op no:

Age: Sex:

Address: Phone no:

Chief complaint:

Intra oral examination:

Gingival recession:

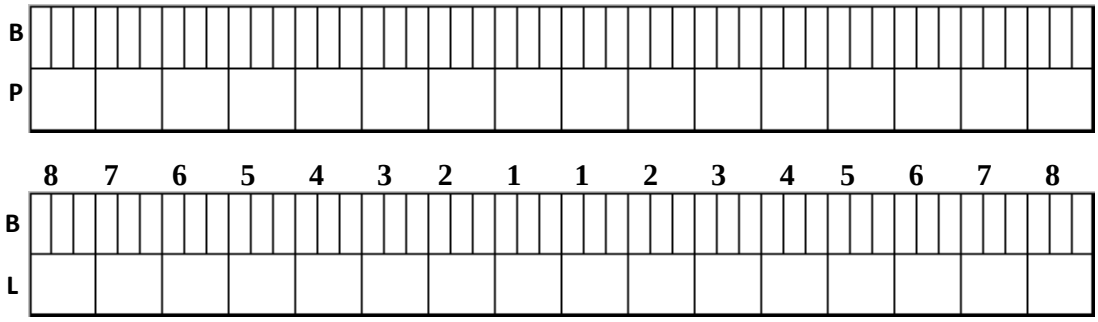
Site selected:

Control (CAF): ☐

Test (CAF + PRF): ☐

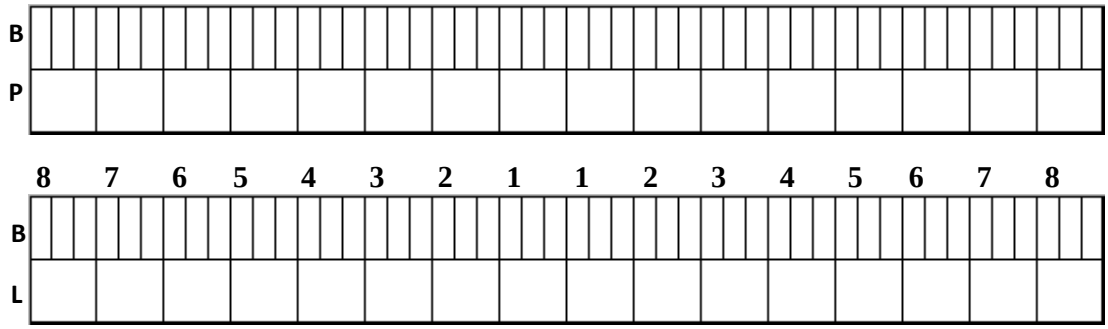
Plaque Index (PI) ***(Silness and Loe 1964)***

Baseline



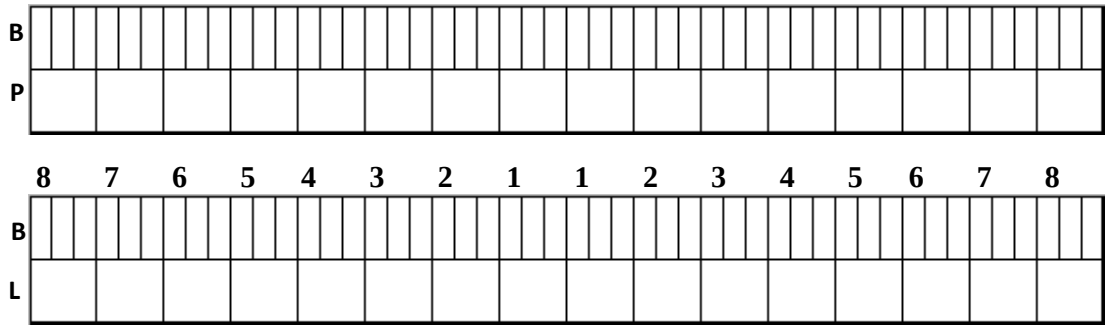
Score

3rd month



Score

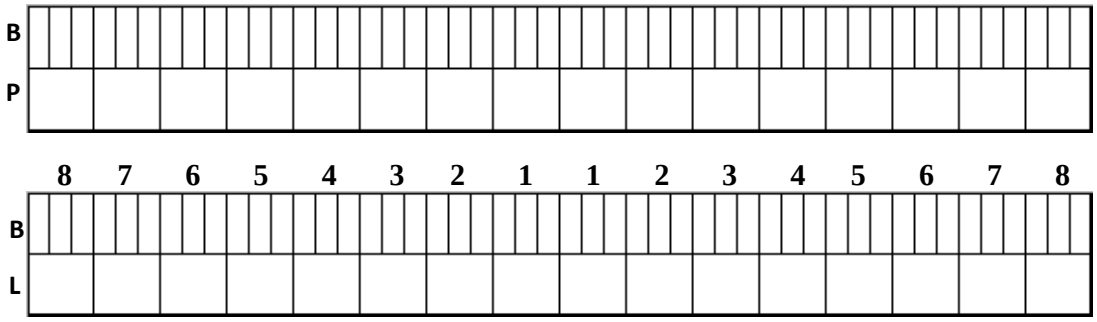
6th month



Score

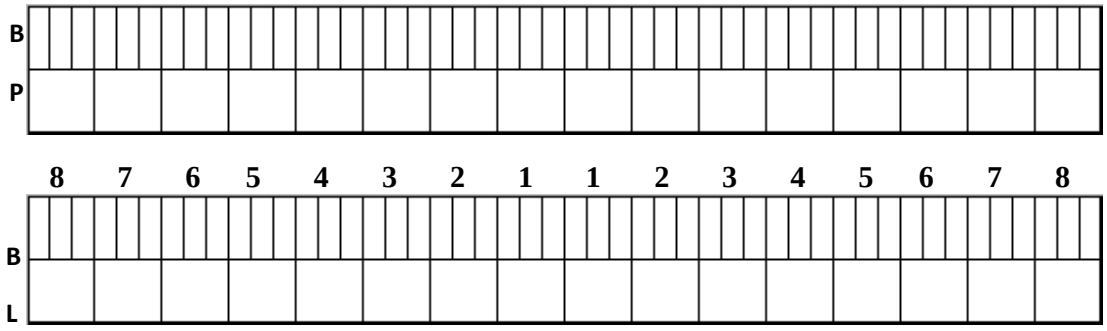
Gingival Index (GI) ***(Loe and Silness 1963)***

Baseline



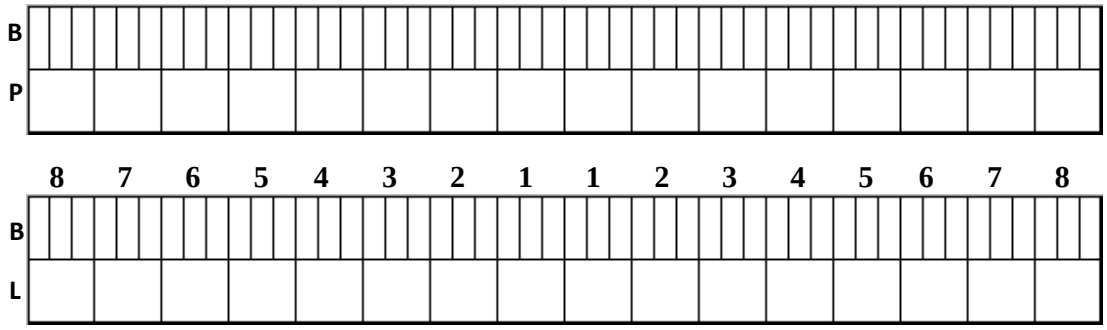
Score

3rd month



Score

6th month



Score

Clinical Parameters

Parameter (mm)	**Baseline**	**3rd month**	**6th month**
Recession depth (RD)			
Recession width (RW)			
Probing depth (PD)			
Clinical attachment level (CAL)			
Width of keratinized tissue (WKT)			
Gingival thickness (GTH)			

INFORMED CONSENT OBTAINED FROM THE PATIENT

Department of Periodontics, JKK Nattraja Dental College, Komarapalayam, Namakkal district, Tamilnadu.

Patient name:

I have been explained about the nature and purpose of the study in which, I have been asked to participate. I understand that I am free to withdraw my consent and discontinue at any time without prejudice to me or effect on my treatment.

I have been given the opportunity to question about the material and study. I have also given the consent for photographs to be taken at the beginning, during and end of the study. I agree to participate in this study.

I hereby give the consent to be included in "Comparative clinical evaluation of coronally advanced flap (CAF) with or without platelet rich fibrin (PRF) membrane in the treatment of isolated gingival recessions."

Station: Signature of the patient

Date: Signature of the Professor

APPENDIX -3

ARMAMENTARIUM

Diagnostic Instruments

- Mouth mirror
- William's periodontal probe
- Acrylic stent
- Gauge
- Endodontic file (number 15)

Surgical Instruments

- Betadine
- 2 % lignocaine HCl with 1: 100000 epinephrine
- Saline
- 3 ml disposable syringe
- 10 ml saline irrigation syringe
- Sterile gauze
- Sterile cotton roll
- Bard parker handle no.3

- Bard parker blade no. 15
- Periosteal elevator
- Gracey curette (Hu-Friedy®)
- Curved Goldman fox scissors
- Tissue holding forceps
- Needle holder
- Kidney tray
- 4-0 non-resorbable suture (MERSILK®)
- Periodontal dressing (Coe-pak®)

Materials and Instruments for PRF Collection

- 10 ml syringe
- Tourniquet
- Glass test tubes
- Centrifuge (R8C Laboratory Centrifuge®, Remi Equipments, Mumbai)

ARMAMENTARIUM

Surgical instruments

Blood collection kit

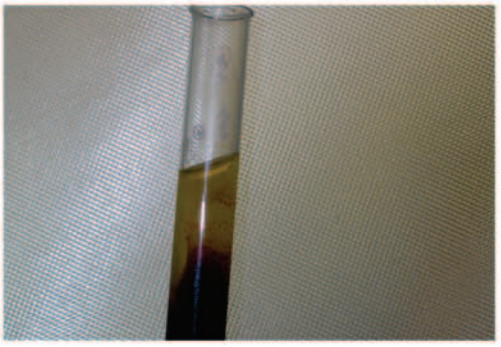
Table top centrifuge

Instruments for PRF collection

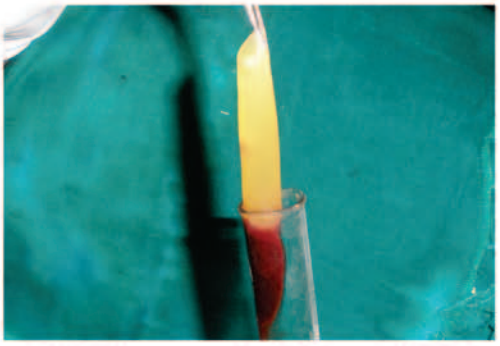
PRF PREPERATION

Blood sample collection

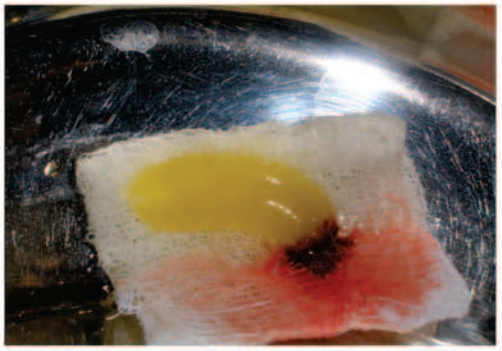
Immediate centrifugation



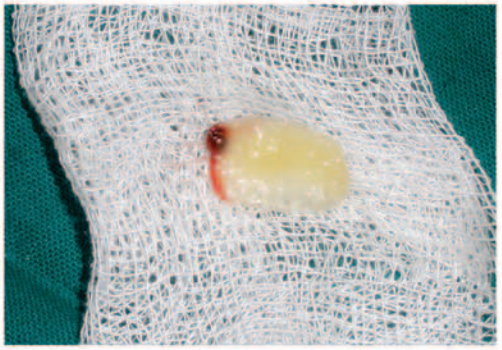
Serum (Top), PRF (middle), RBC (bottom)



PRF seperated from RBC



Soft exudation of serum



PRF membrane

CASES - CONTROL GROUP (CAF)

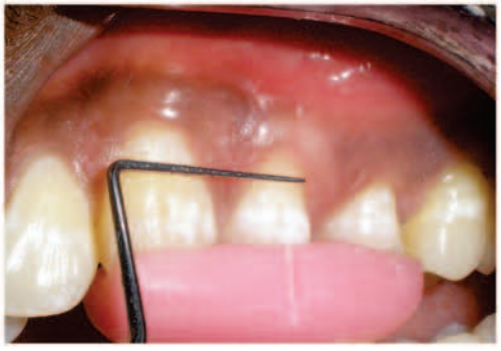
Case – 1 (Tooth 24) Pre operative evaluation

Millers's class I

Recession depth

Clinical attachment level

Width of keratinized tissue



Recession width

Gingival thickness

Operative view

Flap elevation

Flap repositioned & sutured

Periodontal dressing placed

Post operative

1st month

3rd month

Complete Coverage

Clinical attachment level

Width of keratinized tissue

Gingival thickness

6th month

Case - 2 (Tooth 11)

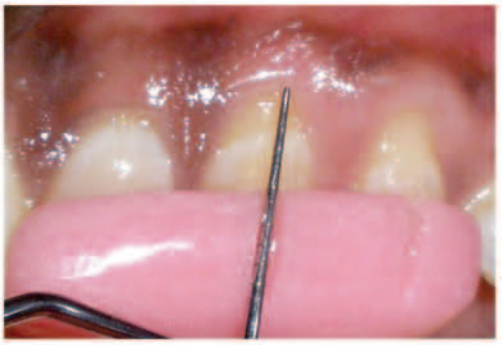
Pre operative

Post operative 6th Month

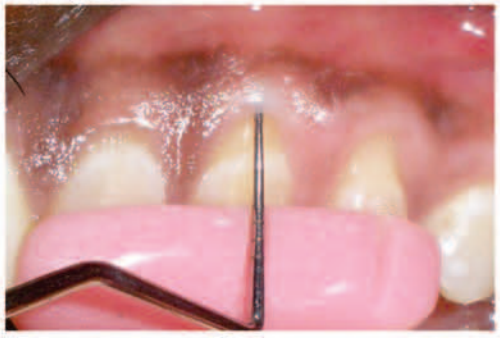
CASES - TEST GROUP (CAF + PRF)

Case – 1 (Tooth 23) Pre operative evaluation

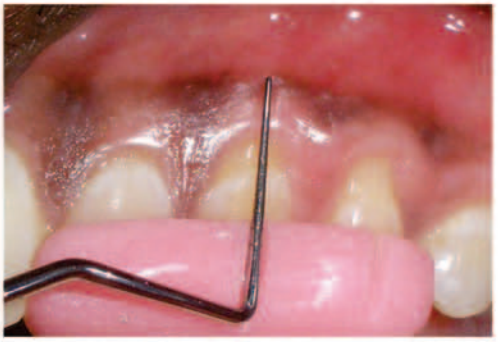
Millers's class I



Recession depth



Clinical attachment level



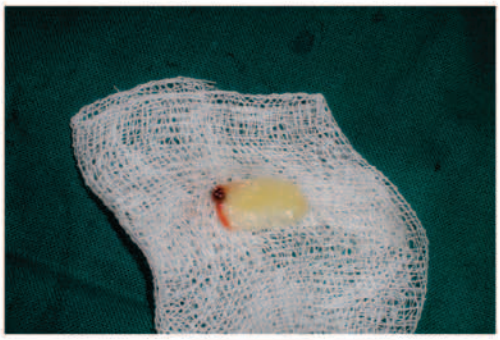
Width of keratinized tissue

Recession width

Gingival thickness

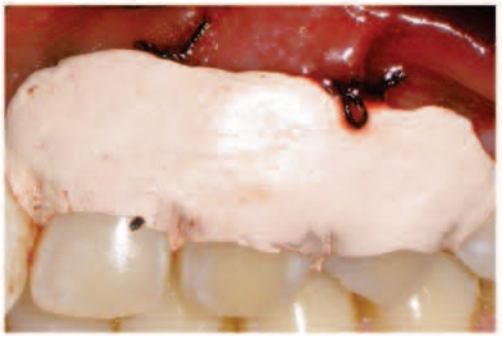
Operative view

Flap elevation



PRF membrane placed over recession defect

Flap repositioned & sutured



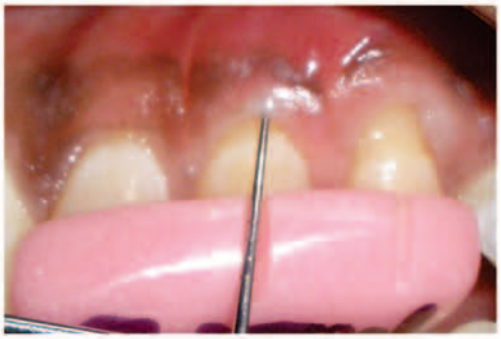
Periodontal dressing given

Post operative

1st month

3rd month

Complete Coverage



Clinical attachment level

Width of keratinized tissue

Gingival thickness

6th month

Case - 2 (Tooth 33)

Pre operative

Flap elevation

PRF membrane placed

Suturing of flap

Post operative 6th month

COMPARISON BETWEEN GROUPS

Control Group (CAF)

Pre Operative

Test Group (CAF + PRF)

Pre Operative

Post Operative 6th Month

Post Operative 6th Month

STATISTICAL METHODS APPLIED

Independent-Samples T Test

The Independent-Samples T Test procedure compares means for two groups of cases. Ideally, for this test, the subjects should be randomly assigned to two groups, so that any difference in response is due to the treatment (or lack of treatment) and not to other factors.

Paired-Samples T Test

The Paired-Samples T Test procedure compares the means of two variables for a single group. It computes the differences between values of the two variables for each case and tests whether the average differs from 0.

All the statistical methods were carried out through the **SPSS for Windows (version 16.0)**

A total of 20 sites were treated, 10 sites each in control and test group. All patients completed the study.

Plaque index (PI)

In control group, the mean PI scores at baseline was 0.89 ± 0.36 and reduced to 0.63 ± 0.22 at 3rd month and 0.55 ± 0.18 at 6th month. In test group, at baseline it was 0.87 ± 0.43 and reduced to 0.60 ± 0.28 at 3rd month and 0.49 ± 0.19 at 6th month. The values at 6th month, were statistically significant (P<0.05) when compared to baseline within the group as shown in Table 1 and Graph 1.

When compared between the groups at baseline, 3rd and 6th month it was not statistically significant (P>0.05) as shown in Table 1 and Graph 1.

Gingival index (GI)

The mean GI score, in control group, at baseline was 0.79 ± 0.36 and reduced to 0.57 ± 0.15 at 3rd month and 0.53 ± 0.17 at 6th month. In test group, at baseline it was 0.87 ± 0.36 and reduced to 0.56 ± 0.21 at 3rd month and to 0.48 ± 0.19 at 6th month. The 6th month values were statistically significant (P<0.05) when compared to baseline within the group as shown in Table 2 and Graph 2.

There was no statistically significant (P>0.05) difference between the groups at baseline, 3rd and 6th month as shown in Table 2 and Graph 2.

Recession depth (RD)

In control group, mean RD at baseline was 2.20 ± 0.91 mm and reduced to 0.70 ± 0.94 mm at 3rd month and 0.90 ± 0.99 mm at 6th month. In test group, at baseline it was 2.30 ± 0.67 mm and reduced to 0.70 ± 0.94 mm at 3rd and 6th month. The values at 6th month were statistically significant ($P<0.05$) when compared to baseline within group as shown in Table 3 and Graph 3.

When compared between the groups at baseline, 3rd and 6th month it was not statistically significant ($P>0.05$) as shown in Table 3 and Graph 3.

Recession width (RW)

The mean RW in control group at baseline was 3.50 ± 0.84 mm and reduced to 1.30 ± 1.70 mm at 3rd month and 1.40 ± 1.64 mm at 6th month. In test group it was 3.40 ± 0.69 mm at baseline and reduced to 1.50 ± 1.64 mm at 3rd and 6th month. The 6th month values were statistically significant ($p<0.05$) when compared to baseline within the group as shown in Table 4 and Graph 4.

There was no statistically significant difference ($p>0.05$) between the groups at baseline, 3rd and 6th month as shown in Table 4 and Graph 4.

Probing depth (PD)

The mean PD at baseline was 1.30 ± 0.48 mm and 1.40 ± 0.51 mm for control and test group respectively and reduced to 1.00 ± 0.00 mm at 3rd and 6th month. The values at 6th month in **test** group were statistically significant ($p<0.05$) when compared to baseline as shown in Table 5 and Graph 5.

When compared between the groups at baseline, 3rd and 6th month, it was not statistically significant (P>0.05) as shown in Table 5 and Graph 5.

Clinical attachment level (CAL)

In control group, mean CAL at baseline was 3.50 ± 0.97 mm and reduced to 1.30 ± 1.33 mm at 3rd and 1.70 ± 1.25 mm at 6th month. In test group, at baseline it was 3.70 ± 0.82 mm and reduced to 1.20 ± 1.39 mm at 3rd and 6th month. The values at 6th month were statistically significant (p<0.05) when compared to baseline within the group as shown in Table 6 and Graph 6.

When compared between the groups at baseline, 3rd and 6th month, it was not statistically significant (P>0.05) as shown in Table 6 and Graph 6.

Width of keratinized tissue (WKT)

Mean WKT in control group, at baseline was 2.40 ± 0.69 mm and reduced to 2.80 ± 0.91 mm at 3rd and 6th month. In test group, at baseline it was 2.30 ± 0.82 mm and reduced to 3.00 ± 0.81 mm at 3rd month and 2.70 ± 0.67 mm at 6th month. The values at 6th month were not statistically significant (p>0.05) when compared to baseline within the group as shown in Table 7 and Graph 7.

There was no statistically significant (P>0.05) difference between the groups at 6th month, as shown in Table 7 and Graph 7.

Gingival thickness (GTH)

The mean GTH in control group was 0.93 ± 0.18 mm at baseline, 0.97 ± 0.18 mm at 3rd month and 0.96 ± 0.18 mm at 6th month. In test group, at the baseline it was 0.95 ± 0.14 mm, and increased to 1.27 ± 0.26 mm at 3rd month and 1.25 ± 0.23 mm at 6th month. The values at 6th month in the **test** group were statistically significant ($p<0.05$) when compared to the baseline as shown in Table 8 and graph 8.

When compared between the groups at 3rd and 6th month it was statistically significant ($p<0.05$) as shown in Table 8 and Graph 8.

Mean root coverage (%) (MRC)

In control group, the mean root coverage was 70.83 ± 42.89 % at 3rd month and 65.00 ± 44.47 % at 6th month. In test group, it was 74.16 ± 28.98 % at 3rd and 6th month as shown in Table 9 and Graph 9.

There was no statistically significant ($P>0.05$) difference between the groups at 3rd and 6th month as shown in Table 9 and Graph 9.

Complete root coverage (CRC)

In control group, complete root coverage occurred in 60 % of the treated sites at 3rd month, and 50 % at 6th month. In test group it was 50 % at 3rd and 6th month as shown in Table 10 and Graph 10.

There was no statistically significant ($P>0.05$) difference between the groups at 3rd and 6th month as shown in Table 10 and Graph 10.

Table 1: Comparison of mean Plaque index (PI) between groups at baseline, 3rd and 6th month

Parameter		Control	Test	*P*
Plaque Index (PI)	Baseline	0.89 ± 0.36	0.87 ± 0.43	> 0.05
	3rd month	0.63 ± 0.22	0.60 ± 0.28	> 0.05
	6th month	0.55 ± 0.18†	0.49 ± 0.19†	> 0.05

† Within group comparison (p<0.05)

Table 2: Comparison of mean Gingival index (GI) between groups at baseline, 3rd and 6th month

Parameter		Control	Test	*P*
Gingival Index (GI)	Baseline	0.79 ± 0.36	0.87 ± 0.36	> 0.05
	3rd month	0.57 ± 0.15	0.56 ± 0.21	> 0.05
	6th month	0.53 ± 0.17†	0.48 ± 0.19†	> 0.05

† Within group comparison (p<0.05)

Table 3: Comparison of mean Recession Depth (RD) between groups at baseline, 3rd and 6th month

Parameter		Control	Test	*P*
Recession Depth (RD)	Baseline	2.20 ± 0.91	2.30 ± 0.67	> 0.05
	3rd month	0.70 ± 0.94	0.70 ± 0.94	> 0.05
	6th month	0.90 ± 0.99†	0.70 ± 0.94†	> 0.05

† Within group comparison (p<0.05)

Table 4: Comparison of mean Recession Width (RW) between groups at baseline, 3rd and 6th month

Parameter		Control	Test	*P*
Recession Width (RW)	Baseline	3.50 ± 0.84	3.40 ± 0.69	> 0.05
	3rd month	1.30 ± 1.70	1.50 ± 1.64	> 0.05
	6th month	1.40 ±1.64†	1.50 ± 1.64†	> 0.05

† Within group comparison (p<0.05)

Table 5: Comparison of mean Probing Depth (PD) between groups at baseline, 3rd and 6th month

Parameter		Control	Test	*P*
Probing Depth (PD)	Baseline	1.30 ±0.48	1.40 ± 0.51	> 0.05
	3rd month	1.00 ± 0.00	1.00 ± 0.00	> 0.05
	6th month	1.00 ± 0.00	1.00 ± 0.00†	> 0.05

† Within group comparison (p<0.05)

Table 6: Comparison of mean Clinical Attachment Level (CAL) between groups at baseline, 3rd and 6th month

Parameter		Control	Test	*P*
Clinical Attachment Level (CAL)	Baseline	3.50 ± 0.97	3.70 ± 0.82	> 0.05
	3rd month	1.30 ± 1.33	1.20 ± 1.39	> 0.05
	6th month	1.70 ± 1.25†	1.20 ± 1.39†	> 0.05

† Within group comparison (p<0.05)

Table 7: Comparison of mean Width of Keratinized Tissue (WKT) between groups at baseline, 3rd and 6th month

Parameter		Control	Test	*P*
Width of Keratinized Tissue (WKT)	Baseline	2.40 ± 0.69	2.30 ± 0.82	> 0.05
	3rd month	2.80 ± 0.91	3.00 ± 0.81	> 0.05
	6th month	2.80 ± 0.91	2.70 ± 0.67	> 0.05

† Within group comparison (p<0.05)

Table 8: Comparison of mean Gingival Thickness (GTH) between groups at baseline, 3rd and 6th month

Parameter		Control	Test	*P*
Gingival Thickness (GTH)	Baseline	0.93 ± 0.18	0.95 ± 0.14	> 0.05
	3rd month	0.97 ± 0.18	1.27 ± 0.26	< 0.01*
	6th month	0.96 ± 0.18	1.25 ± 0.23†	< 0.01*

† Within group comparison (p<0.05) * Between group comparison (*p*<0.05)

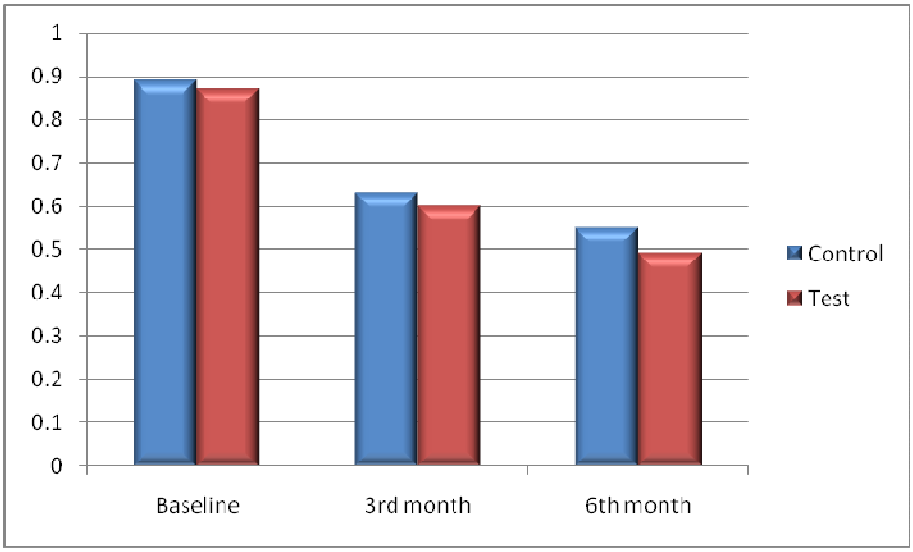
Table 9: Comparison of percentage of Mean Root Coverage (MRC) between groups at 3rd and 6th month

Parameter		Control	Test	*P*
MRC (%)	3rd month	70.83 ± 42.89	74.16 ± 28.98	> 0.05
	6th month	65.00 ± 44.47	74.16 ± 28.98	> 0.05

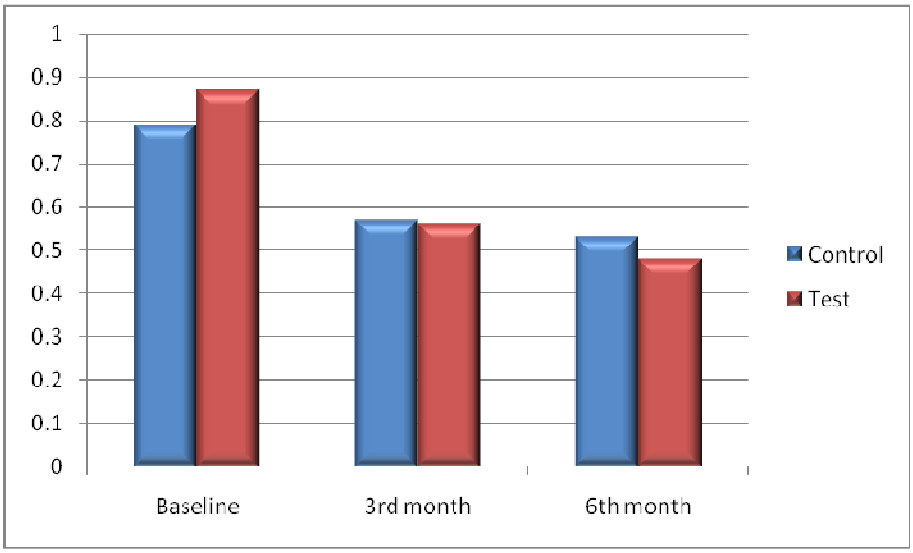
Table 10: Comparison of percentage of Complete Root Coverage (CRC) between groups at 3rd and 6th month

Parameter		Control	Test	*P*
CRC (%)	3rd month	60	50	> 0.05
	6th month	50	50	> 0.05

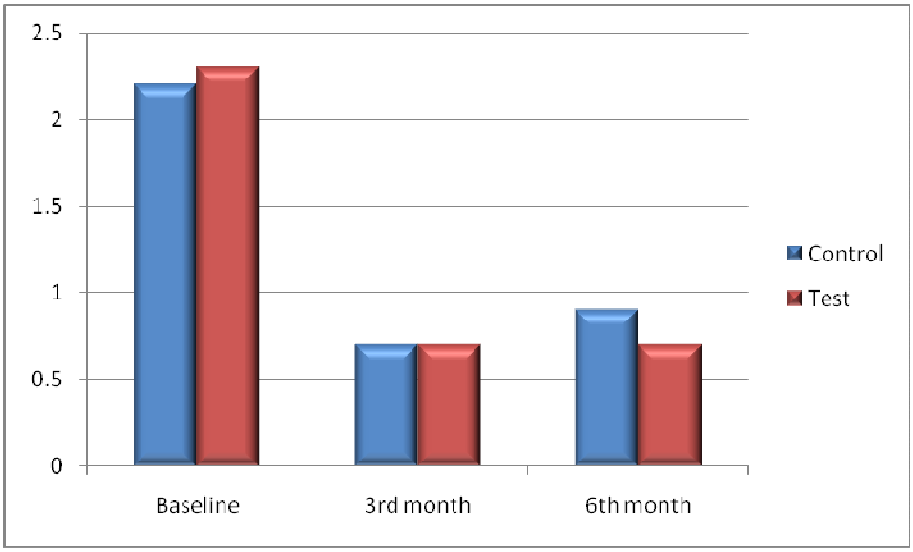
Graph 1: Comparison of mean Plaque index (PI) between groups at baseline, 3rd and 6th month



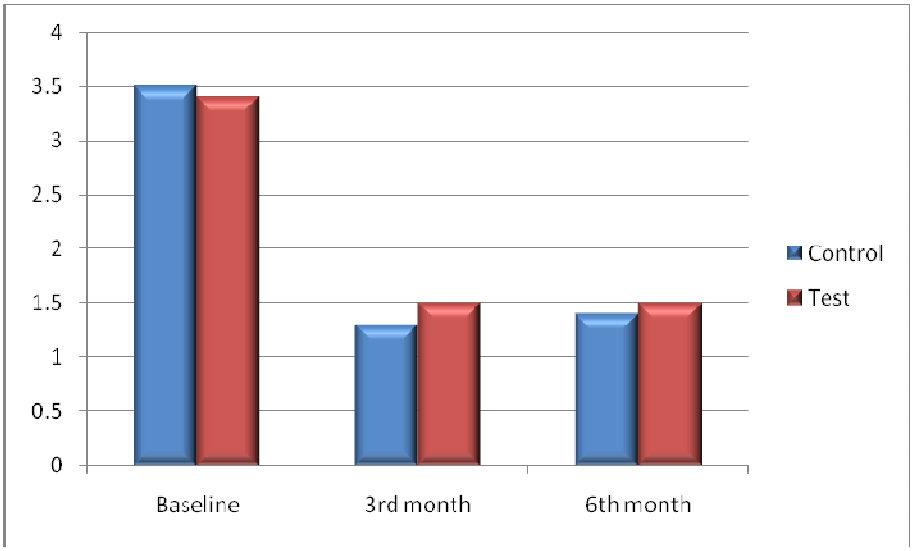
Graph 2: Comparison of mean Gingival Index (GI) between groups at baseline, 3rd and 6th month



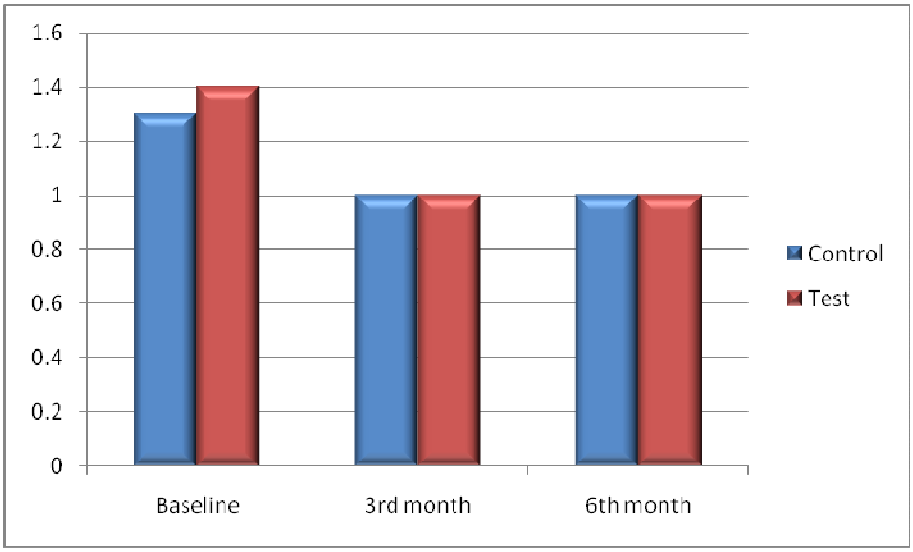
Graph 3: Comparison of mean Recession depth (RD), between groups at baseline, 3rd and 6th month



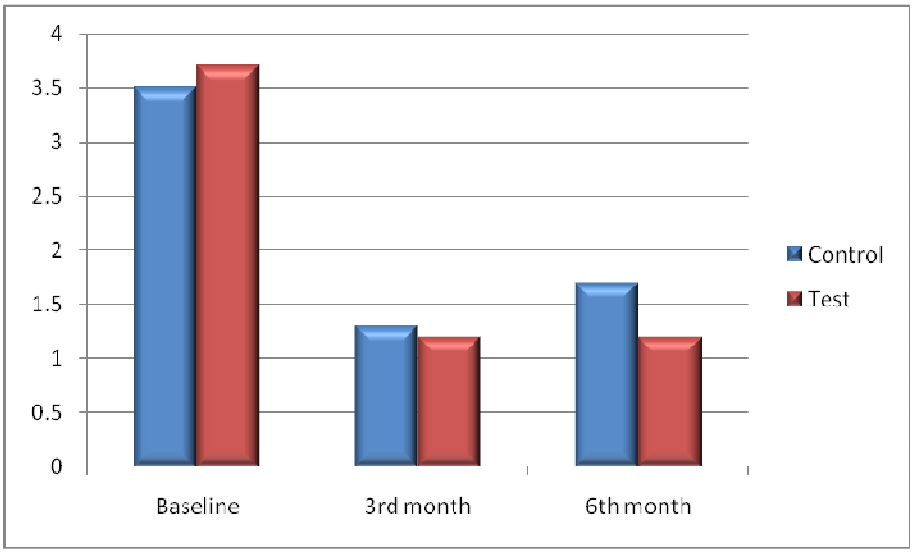
Graph 4: Comparison of mean Recession width (RW), between groups at baseline, 3rd and 6th month



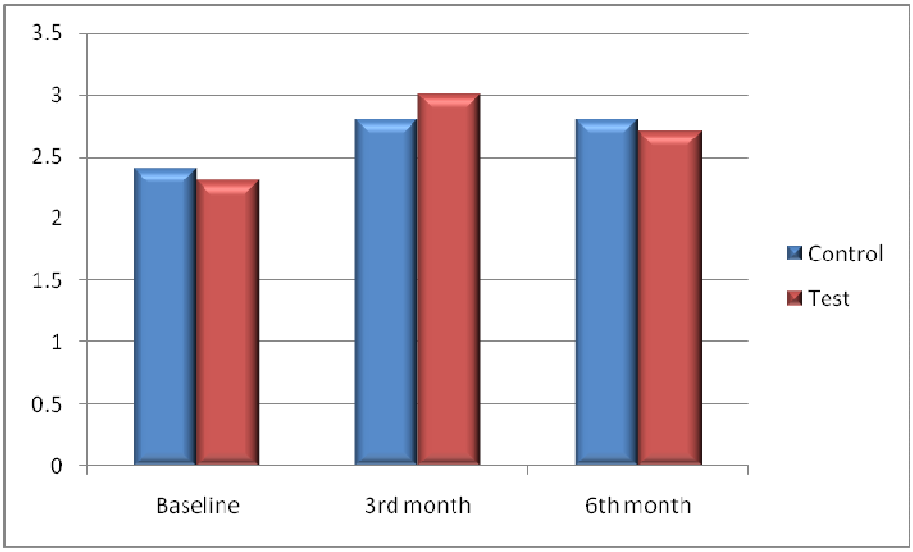
Graph 5: Comparison of mean Probing depth (PD) between groups at baseline, 3rd and 6th month



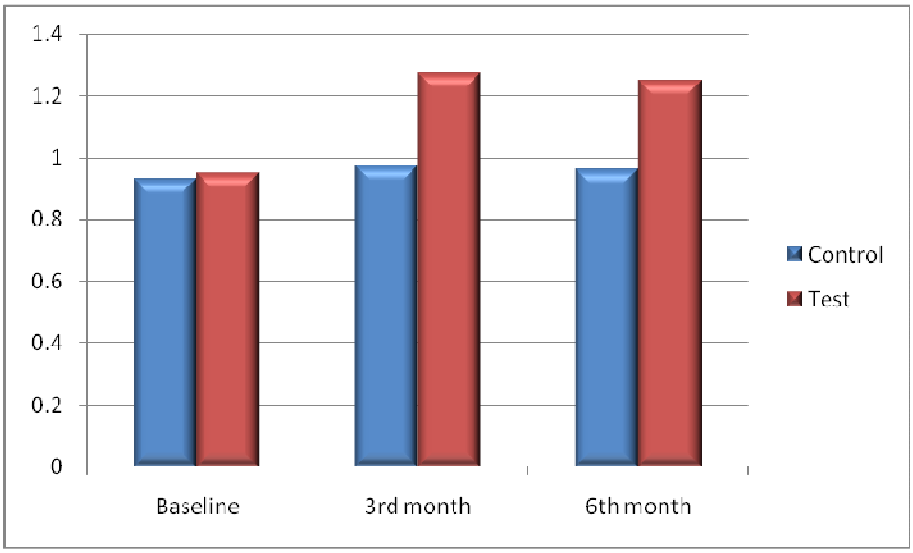
Graph 6: Comparison of mean Clinical attachment level (CAL) between groups at baseline, 3rd and 6th month



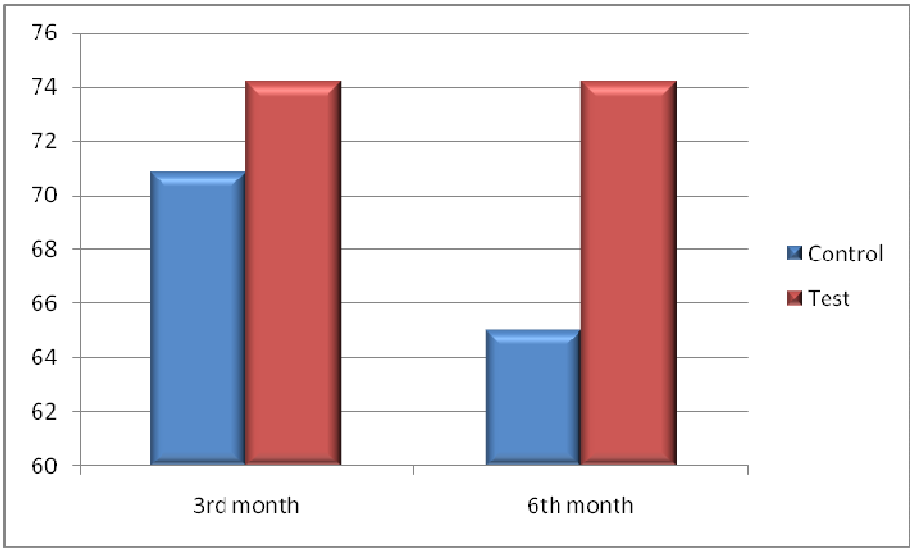
Graph 7: Comparison of mean Width of keratinized tissue (WKT) between groups at baseline, 3rd and 6th month



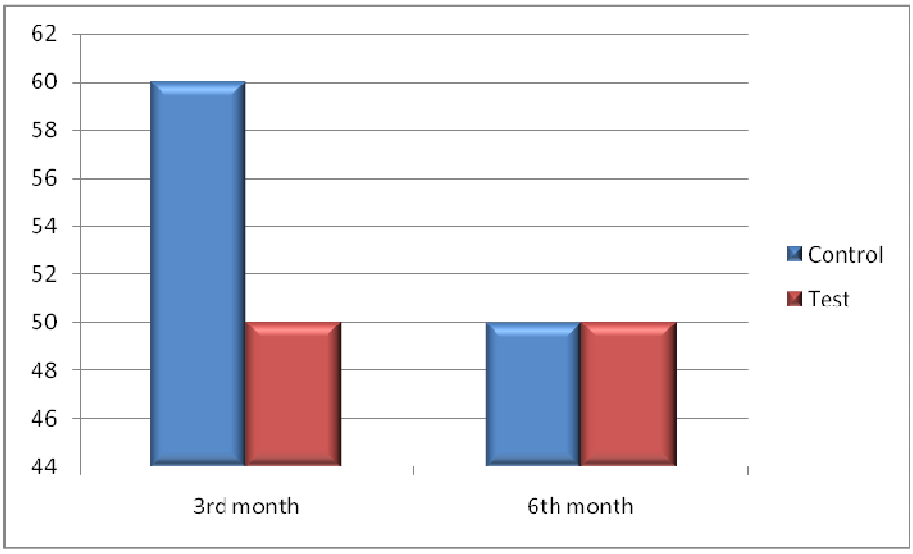
Graph 8: Comparison of mean Gingival thickness (GTH) between groups at baseline, 3rd and 6th month



Graph 9: Comparison of percentage of Mean root coverage (MRC) between groups at baseline, 3rd and 6th month



Graph 10: Comparison of percentage of Complete root coverage (CRC) between groups at baseline, 3rd and 6th month



Historically, periodontal therapy has been directed primarily at the elimination of disease and the maintenance of functional, healthy periodontal tissues.[73] Currently, it is also focused on esthetic root coverage procedures.[73] However, these procedures do not always result in regeneration of attachment apparatus[6] like cementum, periodontal ligament and bone, which is a major risk factor in recurrence of gingival recession.[73] Thus techniques aiming at both regeneration of functional attachment apparatus and root coverage seem to be advantageous.[74] The availability of autologous PRF, to enhance the regenerative process of periodontal tissues has been reported in several studies. [66, 67, 68,75] In addition they are safe from transmission of disease, immune reactions and also appear to enhance the soft and hard tissue healing.[75] Hence, the present study was conducted to clinically evaluate the effectiveness of autologous PRF membrane with CAF in the treatment of isolated gingival recession compared to CAF alone.

In the present study the clinical outcomes were evaluated over a period of 6 months. This is in agreement with ***Cheng YF et al. (2007)[76]*** who stated that a 6 month post operative measurement period is sufficient to evaluate the stability of the gingival margin after a CAF.

The mean PI and GI score in the present study showed a marked improvement ($p<0.05$) for both treatment groups from baseline to 6^{th} month. The changes at 6^{th} month, between the groups were not statistically significant ($p>0.05$). Similar results were reported by ***Woodyard et al. (2004)***[38] and ***Gurgan CA et al. (2004)***[77] who observed a marked reduction of GI and PI, in patients undergoing periodontal

therapy, which may be attributed to the reinforcement of oral hygiene and regular monitoring of the patients.

The mean root coverage in control group was 70.83 % at 3rd month and 65 % at 6th month. Similar results were reported by, ***Lins et al. (2003)***[40] with 60 % and ***Cortes et al. (2004)***[37] with 71 %, using CAF at 6 months follow-up. In the present study the reduction of mean root coverage from 3rd to 6th month is in accordance with ***Pini-Prato et al. (2010)***[36] who observed an apical shift of the gingival margin in CAF treated sites. The estimated average apical shift was 0.024 mm per year.[34] This might be related to the thin marginal tissue and amount of keratinized tissue achieved, leading to possible relapse of gingival margin during the maintenance phase.[36] In addition, resumption of the traumatic tooth brushing habits, in patients with high levels of oral hygiene, even if they were included in a stringent maintenance protocol, could lead to the observed relapse of the soft tissue defects.[34]

The test group showed mean root coverage of 74.16 % at 3rd and 6th month. This is in agreement with ***Jankovic et al. (2010)***,[71] who reported mean root coverage of 72 % using PRF at 12 months follow up. In the present study, stable mean root coverage was maintained between 3rd till the 6th month. This is in accordance with ***Shephard N et al. (2009)***,[55] who observed no change in the mean root coverage obtained postoperatively, between 2nd and 4th month follow ups, when PRP was used. This may suggest that, platelet concentrates promotes more rapid attachment to the tooth with stable result. The present study did not show a statistically significant ($p>0.05$) difference in mean root coverage between the groups at 3rd and 6th month.

Complete root coverage is the most important outcome in patients with esthetic demand. In control group it was obtained in 60 % of sites at 3rd month and 50 % at 6th month. This result concurs with ***Leknes et al. (2005)***[41] who reported complete root coverage in 50 % of the sites treated with CAF at the end of 6th month. In test group it was 50 % at 3rd and 6th month. This is agreement with ***Aroca S et al. (2009)***[70] who reported 52 % in multiple recession defects using CAF + PRF. In the present study there was no statistically significant difference ($p>0.05$) between the groups in terms of complete root coverage.

The mean PD showed a reduction from baseline to 6th month, for both control and test groups. These findings compare well with ***Aroca et al. (2009)***[70] who observed a reduction in probing depth from baseline to 6th month. The reduction is probably due to the close adaptation of the new buccal soft tissue, which is an efficient obstacle for probe penetration.[41] The comparison between groups in the present study showed no statistically significant ($p>0.05$) difference in PD at 6th month.

The control group showed a statistically significant ($p<0.05$) mean CAL gain of 1.8 ± 0.91 mm at 6th month. This is in agreement with ***Cortes et al. (2004)***[37] who reported a 2 mm gain for CPF at 6th month. This observed gain in CAL, may be due to the coronal advancement of flap.[6]

There was a statistically significant ($p<0.05$) mean CAL gain of 2.5 ± 1.17 mm observed in test group. This is in accordance with ***Aroca S et al. (2009)***,[70] where a gain of 2.47 mm in CAF+PRF group for multiple recession defects was reported.

This increase in CAL gain might be attributed to the healing and interpositional property of PRF, as proposed by ***Del Corso et al. (2009)***.[59] PRF as a healing material stimulates the gingival connective tissue on its whole surface, with growth factors and impregnates the root surface with key matrix proteins, for cell migration. Moreover the fibrin matrix itself shows mechanical adhesive properties and biologic functions like fibrin glue, which maintained the flap in a high and stable position, enhances neoangiogenesis, reduces necrosis, resulting in maximum root coverage. As an interpositional matrix PRF layers prevents the early invagination of the gingival epithelium.[59] However, the present study did not show statistically significant ($p>0.05$) difference for CAL gain between the groups at 6th month.

The control group showed an increase in mean WKT by 0.4 mm at 6th month. This is in accordance with ***Cortes et al. (2004)***[37] with 0.4 mm and ***Huang et al. (2005)***[53] with 0.6 mm, for CAF at 6th month. The increase in WKT could be the result of the granulation tissue derived from the PDL[78] or the tendency of the mucogingival line to regain its original position.[79]

Similarly, test group also showed 0.4 mm increase of mean WKT at 6th month, which is greater than 0.17 mm as reported by ***Jankovic et al. (2010)***,[71] for isolated recession defects in CAF+PRF group. Also ***Jankovic et al. (2007)***[80] in another study observed a higher WKT gain, as a result of the influence of growth factors from PRP. The present study did not demonstrate statistically significant ($p>0.05$) difference between the groups for WKT at 6th month.

A 0.03 mm increase ($p>0.05$) of mean GTH was observed in the control group after 3rd and 6th month. This compares well with ***Huang LH et al, (2005)***[53] where a 0.03 mm increase was observed at 6th month for CAF.

The test group showed a 0.30 mm increase in mean GTH from baseline to 6th month, which was statistically significant ($p<0.05$). The increased GTH in test group showed a statistically significant difference ($p<0.05$) when compared to control group at 3rd and 6th month. This is in agreement with ***Aroca S at al. (2009)***[70] who reported a statistically significant increase in GTH, for multiple recession defects using PRF. The increase in soft tissue thickness may be due to the influence of growth factors from PRF membrane on the proliferation of gingival and PDL fibroblasts or to a spacing effect of PRF membrane.[70]

Henderson et al. (2001)[81] hypothesized that the critical determinant of future gingival recession may be marginal gingival thickness, than the width of keratinized tissue. This is in accordance with ***Pini-Prato et al. (2010)***[36] who observed an increased percentage of sites with complete root coverage, due to a thick gingival tissue, which facilitated a creeping attachment between 1 and 5 years of follow-up. However, the proper evaluation of the effect of gingival thickness on root coverage stability (i.e. no change, further recession, or creeping attachment) necessitates more investigations with greater follow–up visits.

Thus, in the present study, both treatment techniques resulted in a favorable clinical outcome in terms of root coverage obtained. While comparing the 2 groups,

there was no statistically significant difference for any of the clinical parameters except for an increase in GTH in the test group.

Factors such as PRF consistency, platelet concentration were not tested in the present study which may have affected the final clinical outcome. In addition, no histologic evaluation was performed to assess the type of healing. Therefore the effect of PRF on establishment of a connective tissue attachment remains to be determined. zz

The present study involved a comparative clinical evaluation of CAF with or without PRF membrane in the treatment of isolated gingival recession. The study population comprised of 20 subjects each with one Miller's class I or II buccal recession defects. After randomization the control group was treated with CAF alone and the test group using CAF combined with PRF membrane. Clinical parameters like Gingival index (GI), Plaque index (PI), Recession depth (RD), Recession width (RW), Probing depth (PD), Clinical attachment level (CAL), Width of keratinized tissue (WKT) and Gingival thickness (GTH) were assessed at baseline, 3rd and 6th month. The data thus obtained were statistically analyzed using SPSS for Windows (version 16.0).

From this randomized, controlled clinical study, the following conclusions have been elucidated,

- CAF with and without the addition of PRF membrane yielded favorable clinical outcome in treating isolated gingival recession.
- When comparing between the groups, there was no additional benefit by combining PRF with CAF in terms of Mean root coverage (MRC), Clinical attachment level (CAL) gain and Width of keratinized tissue (WKT) at 6th month.
- With the addition of PRF membrane to CAF there was a statistically significant ($p<0.01$) increase in Gingival thickness (GTH) at 6th month.

Within the limits of this study, it is important to emphasize that, the increased gingival thickness (GTH) obtained with PRF membrane and its influence on preventing further recession, should be evaluated with the studies involving larger number of samples and longer follow-up periods.

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